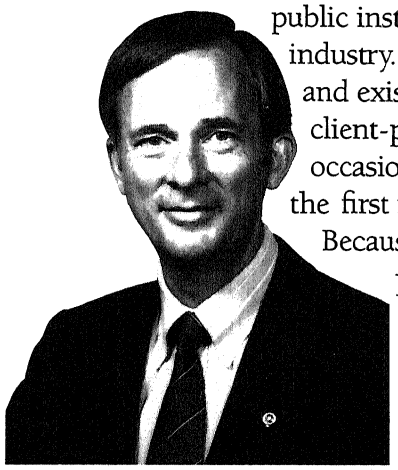
Regional Poultry Research Laboratory

For half a century, the Regional Poultry Research Laboratory (RPRL) has been dedicated to the pursuit of knowledge that will help solve practical poultry disease problems of concern to the poultry industry and governmental regulatory agencies. Effort has focused largely on the transmissible viral neoplasms (cancer-like diseases) of poultry. The research team has endeavored to apply sophisticated technology and fundamental approaches toward practical goals such as the development of vaccines or strains of genetically resistant chickens.

Throughout this work, our scientists have maintained a close relationship with scientists at public institutions and with the poultry industry. It is with recognition of these historic and existing relationships with our client-partners that the RPRL has taken the occasion of its 50th anniversary to produce the first in a series of progress reports. Because of the occasion, this narrative also presents a brief historical review of earlier achievements.



Dr. R.L. Witter
Director

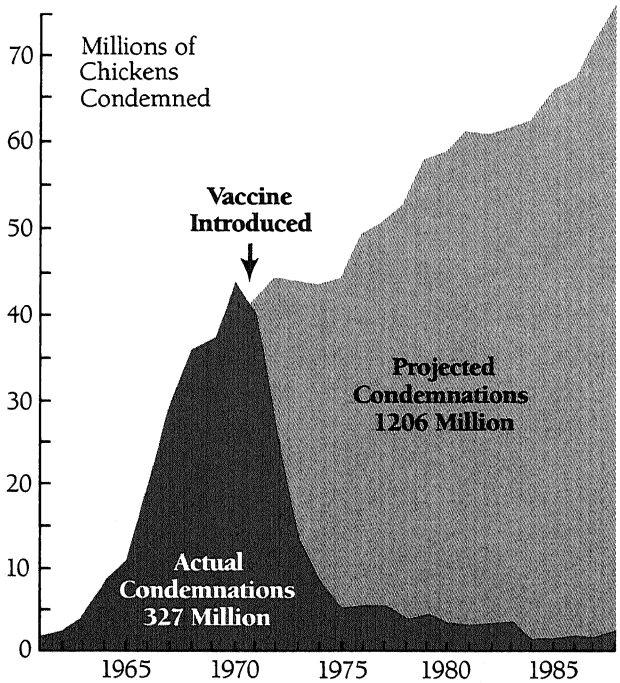
Historical Background

The RPRL was dedicated on August 8, 1939; its establishment was the result of joint efforts by the Agricultural Experiment Stations of the Northeastern and North Central states and the United States Department of Agriculture. The original mission of the RPRL was to conduct research on the improvement of the viability of poultry as mortality in both farm and commercial poultry flocks had increased substantially since about 1925. In many cases, mortality was associated with "fowl paralysis," "big liver disease," and "gray eye," a group of diseases that came to be known as the avian leukosis complex. This complex was to be the main thrust of the Laboratory research.

Several research areas were established. Genetics research was aimed at developing inbred lines of chickens that were resistant or susceptible to the avian leukosis complex and ones which were susceptible but free of the disease for use in pathologic studies. Both of these goals have been achieved and the Laboratory maintains one of the few flocks of highly inbred chickens that are kept free of most common poultry disease agents.

A pathology group was formed to determine the causative agents of the disease and to study disease transmission. Early studies identified a

virus that caused "big liver disease," which is now called lymphoid leukosis, and showed that this virus could be transmitted congenitally through the egg as well as from chick to chick. These were some of the earliest studies of the natural transmission of a cancer virus and received worldwide recognition.



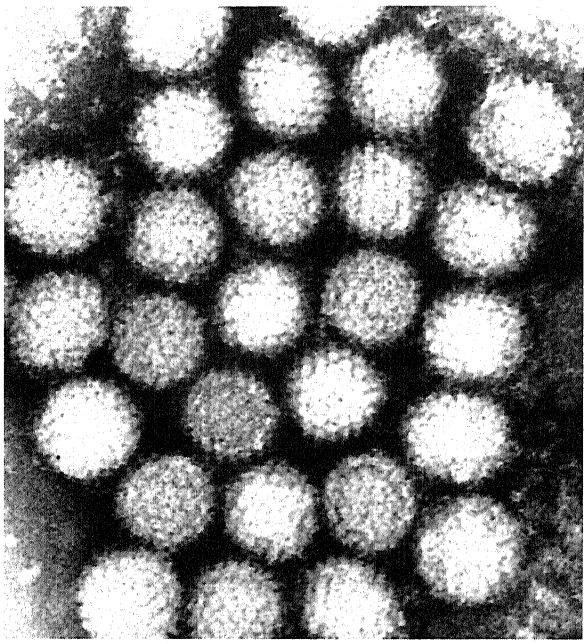
Effect of vaccination on Marek's disease condemnations in broiler chickens in the United States. Projected total based on prevaccination condemnation rate.

It was soon realized that the microscopic and gross anatomy of the chicken was poorly understood and that a better understanding of anatomical structure of the fowl would facilitate the disease studies. Research in this area led to some of the most definitive studies on avian hematology and anatomy.

It became apparent that a great deal of basic information was needed before practical control measures for avian leukosis could be developed and the first 25 years were devoted to a gradual accumulation of this knowledge. Little information on practical control of these diseases was developed during this period and the farmer lived with chronic mortality while commercial breeders gradually developed strains of chickens with increased viability.

The 1960's brought several changes. The cancer research community became very interested in virus-induced cancer and in the possibility that viruses could cause cancer in man. The chicken became an ideal model for this research, and techniques for studies of the viruses causing lymphoid leukosis became much more rapid and sensitive. At the same time, the broiler industry was expanding rapidly and high rates of mortality and condemnations from tumors in young chickens became devastating. It was soon realized that these

tumors were manifestations of a condition called Marek's disease after the Hungarian pathologist who first described it in 1907. At this time, most of the research at the Laboratory was devoted to the Marek's disease problem. The causative herpesvirus was identified and it was found that



Electronmicrograph of Marek's disease virus.

a related virus of turkeys could be used as an effective vaccine; this vaccine is still used for the control of Marek's disease worldwide.

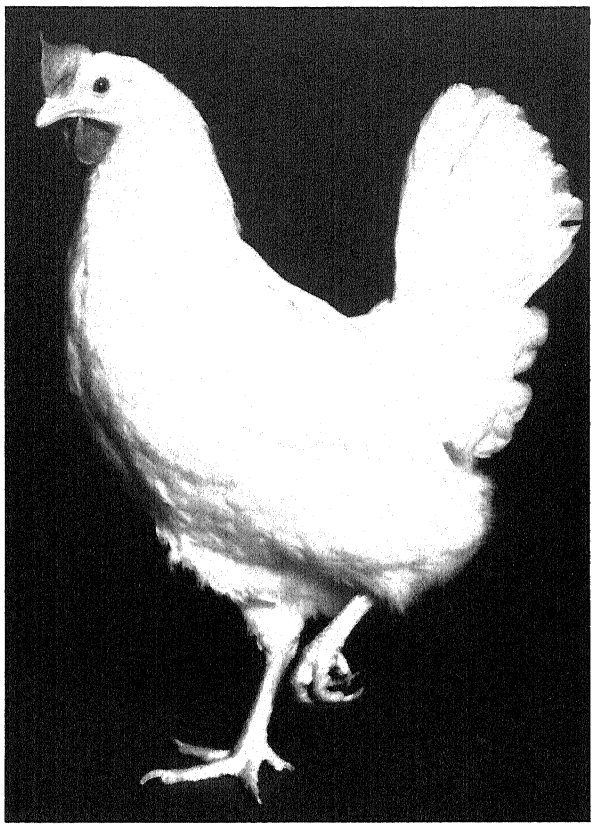
During the early 1970's, a new wing was added to the main Laboratory and chicken isolation facilities were remodeled so that studies of the various diseases could be conducted without cross contamination. All the breeding stock was reared free of the major poultry pathogens. Following the development of the Marek's disease vaccine, the Laboratory efforts became more balanced. Some of the effort was refocused on the study of lymphoid leukosis and the development of methods for the control of its causative virus. Methods were developed to break the cycle of maternal transmission, and when applied in the field, led to a substantial reduction in virus infection. Work on hemorrhagic enteritis and infectious bursal disease was also initiated, as well as research on general aspects of disease resistance in poultry.

Mission and Organization

The current research program of the RPRL is still focused on the transmissible viral neoplasms of poultry, particularly lymphoid leukosis, Marek's disease, and reticuloendotheliosis. However, the program also includes further areas of investigation to control other viral diseases, to identify genes influencing immune response, to insert foreign genes into the chicken germ line, and to produce viral vectors for use in genetically engineered vaccines. Results of these studies benefit broiler, layer and breeder chickens as well as turkeys and other poultry.

In order to utilize the multidisciplinary expertise available at the RPRL in the fields of genetics, immunology, molecular biology, virology, and veterinary medicine, informal research teams have been created, each with their own project goals but with the prospect of interactions with other teams. Projects have typically been basic, high risk, and long term, but with the goal of solving practical problems for the poultry industry and regulatory agencies.

A priority has been placed on a team approach, not only among laboratory scientists but with cooperators at neighboring Michigan State University, other institutions, and with poultry breeding and poultry biologics companies.



The research team, varying from 7 to 15 scientists plus up to 30 support persons, has produced over 620 publications in scientific journals and is internationally recognized for its contributions to biological science and to poultry health.

Leukosis

avian leukosis virus causes
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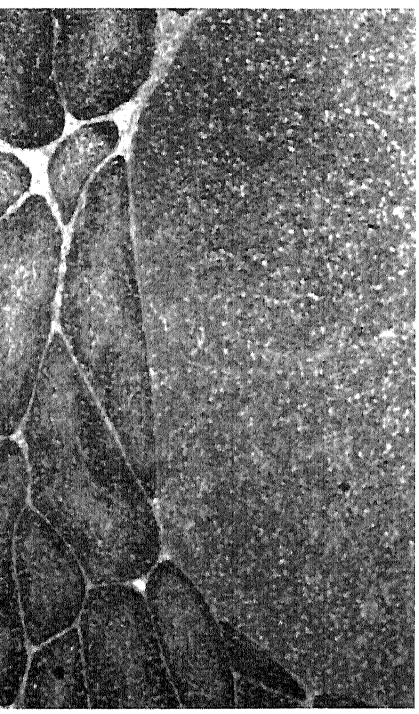

rance of a tumorous follicle surrounded by the bursa of Fabricius, the target organ for

controlling the infection in the field. Recent research has identified conditions of environmental exposure and certain host genes that can influence virus infection and shedding. The research also revealed that in certain lines of chickens, lymphoid leukosis can be enhanced by serotype 2 Marek's disease vaccines. Control of virus infection in progeny chicks by induction of maternal antibody through vaccination of breeders with noninfectious vaccines is now under investigation.

Reticuloendotheliosis

Research on reticuloendotheliosis, another virus-induced neoplastic disease primarily of turkeys, has focused on development of highly specific monoclonal antibodies and more sensitive assays for detection of the virus. These assays and reagents have been very useful in studying the pathogenesis and epidemiology of the disease in chickens and turkeys.

Endogenous Virus

An endogenous virus gene linked to the sex-linked slow-feathering gene (*K*) has been shown to code for a complete endogenous avian leukosis virus. Research has shown that feather-sexed chickens congenitally infected with this endogenous virus are immunologically tolerant toward infection by exogenous

Research at the RPRL demonstrated a close linkage between an endogenous avian leukosis virus gene and the gene determining slow-feathering.

oncogenic strains of avian leukosis viruses. Current research is aimed at strategies for eliminating potential detrimental influences of endogenous viruses on infection and shed of exogenous avian leukosis viruses, partic in feather-sexed chickens.

Immunogenetics

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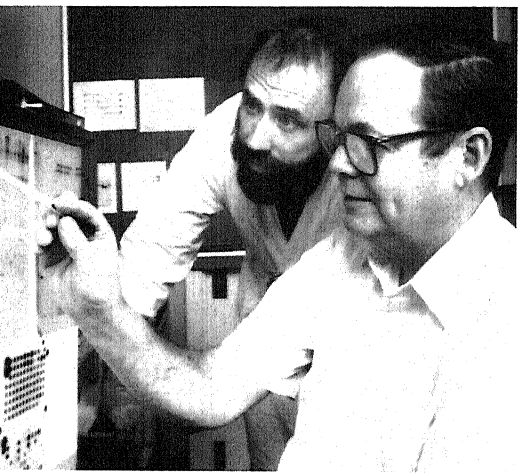

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exogenous oncogenic strains of the avian leukosis virus has been created. Current plans are to use recombinant avian leukosis viruses, disabled by gene-splicing, as vectors for transferring other genes related to disease resistance.

Marek's Disease

Marek's disease in chickens has been largely contained by vaccination, but sporadic vaccine breaks resulting in significant economic losses continue to occur worldwide. Recent research has been aimed principally at developing more efficacious vaccines and a more effective system for vaccine delivery and at gaining a basic understanding of the molecular mechanism involved in pathogenesis of the disease, vaccine immunity and structure, function, and expression of the viral genome. Recently, several new strains of Marek's disease virus have been identified as candidates for improved bivalent or monovalent vaccines. The development of monoclonal antibodies to the virus was instrumental in the identification of genes involved in virus replication and cell transformation.

Monoclonal antibodies developed at the RPRL have been invaluable in the antigenic characterization of strains of Marek's disease virus.

Viral Vectors

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Lymphoid Leukosis

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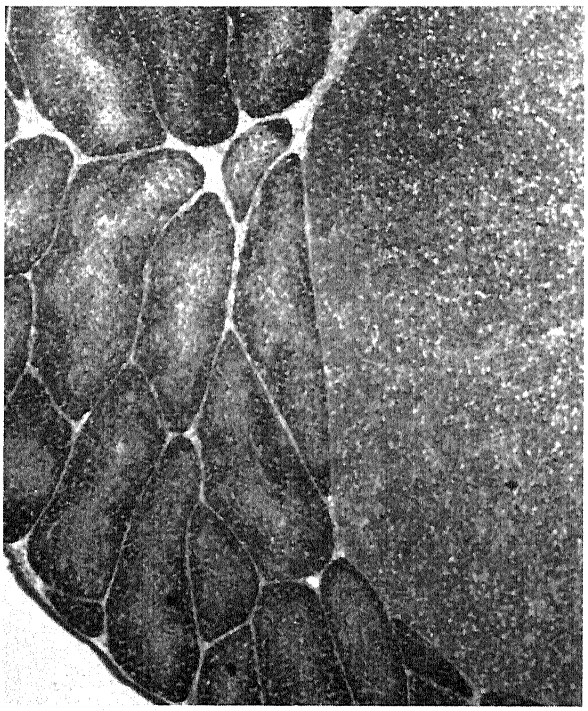

Microscopic appearance of a tumorous follicle surrounded by normal follicles in the bursa of Fabricius, the target organ for lymphoid leukosis.

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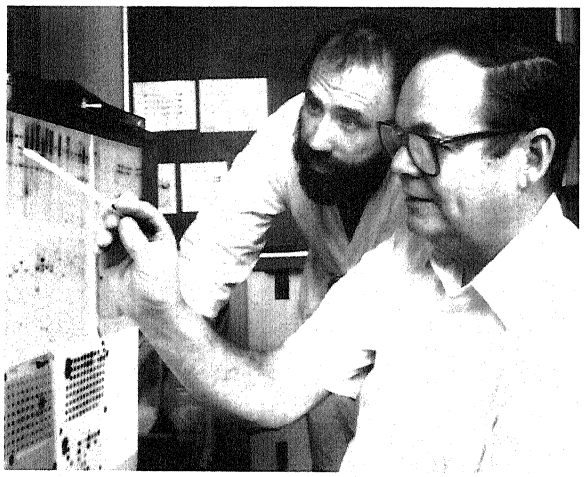
Gene Manipulation

Avian leukosis viruses replicate through integration into the host chromosomes. Recent research has shown that these viruses, modified by gene-splicing methods, can integrate into reproductive cells of the chicken and are



Screening of DNA fragments for the insertion of an avian leukosis virus gene.

inherited in a Mendelian manner. In some chickens, only some of the viral genes are active, but their products can block further infection by the virus. Such chickens transmit resistance to their progeny. By this means, a uniformly resistant line of chickens that is resistant to exogenous oncogenic strains of the avian leukosis virus has been created. Current plans are to use recombinant avian leukosis viruses, disabled by gene-splicing, as vectors for transferring other genes related to disease resistance.

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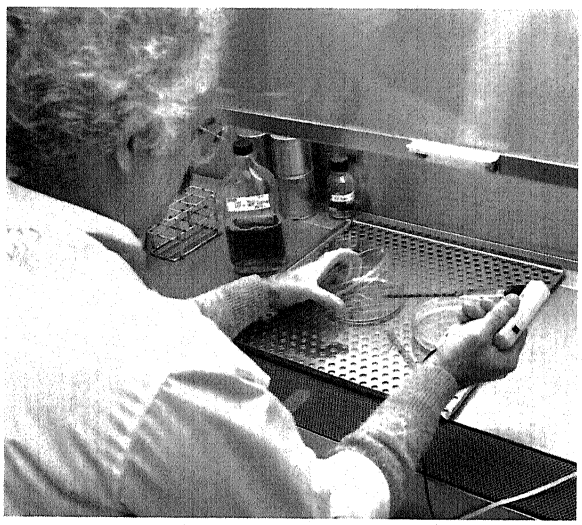
Viral Vectors

Recently, a research project has been initiated to utilize viral vectors to construct new and more efficacious poultry vaccines. One approach has been to modify Marek's disease virus. Toward this goal, a Marek's disease virus replicon (defective virus) to deliver and express foreign genes has been developed. This replicon was used to construct a stable recombinant virus. To optimally regulate the expression of inserted foreign genes, efforts are also being directed toward assaying and ranking the strength of various promoters.

Another approach has been to utilize fowlpox virus. The thymidine kinase gene of fowlpox virus has been cloned, sequenced, and is being used as an insertion site for foreign genes. Efforts will be made to construct recombinant viral vectors that efficiently express genes from various avian pathogens.

Hemorrhagic Enteritis

Hemorrhagic enteritis of turkeys and marble spleen disease of pheasants are caused by antigenically related type II avian adenoviruses. Both viruses were adapted to grow *in vitro* using a turkey lymphoblastoid cell line. Recently, microneutralization and more sensitive enzyme immunoassays were developed for detection of antigen and antibody. Cell-culture-propagated nonpathogenic strains of the virus have successfully been used to vaccinate poults against the disease in the laboratory and in the field. A commercial vaccine against this important disease of turkeys based on technology developed at the RPRL is now available.



Cell lines developed at the RPRL were successfully used for the first time to propagate hemorrhagic enteritis virus of turkeys in vitro.

Infectious Bursal Disease

Pathogenic strains of infectious bursal disease virus continue to emerge in commercial poultry flocks despite passive transfer of maternal antibodies and multiple vaccinations with live attenuated or killed vaccines. Current research is aimed at identification of viral genes associated with virulence.

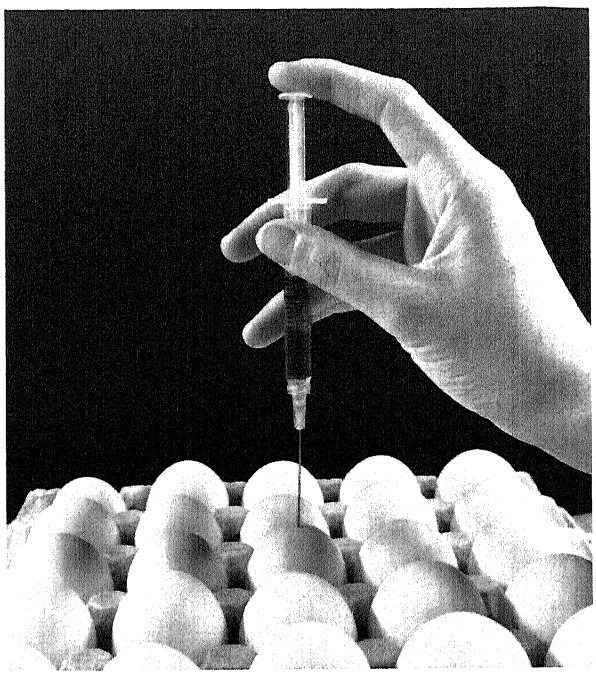
Future Directions

The RPRL embarks on its second half century with pride in past accomplishments and with a sense of renewed dedication to the challenges of providing leadership and research contributions to animal health in an increasingly sophisticated and competitive research environment.

Needs for research on the avian viral tumors continue as the RPRL endeavors to maintain its role as a major, if not the preeminent, research facility capable of addressing this group of economically important and biologically complex diseases.

Increasing emphasis on molecular technologies will make research more complex but will provide new tools needed for contemporary studies. Problems will be addressed through larger and differently structured scientific teams, which may include partnerships with private industry where needed and appropriate. This will require a sharpening of our research focus and creative management of the program.

Challenges from aging facilities and limited budgets are formidable but will be faced forthrightly. Plans are being developed for significant facilities improvements which are necessary to support the planned research program in the coming years. Support in this endeavor, both from the Agricultural Research Service and from our partners in the poultry industry, has been most gratifying.



Embryonic vaccination was shown to be an effective method for immunizing chickens against Marek's disease.

We express our sincere appreciation to those of you who have, and will continue to assist us in our efforts to serve the poultry industry through excellence in science.

Milestones

1947-1954
Viral etiology of lymphoid leukosis was demonstrated and infectious virus was transmitted congenitally and by contact.

1961
An Atlas on Avian Hematology was published.

1963
Host resistance to avian leukosis virus infection was shown to be controlled by a recessive gene in cell-culture and in chickens.

1967
A herpesvirus was identified as the etiologic agent of Marek's disease.

1968-1970
A herpesvirus was isolated from turkeys and shown to be an effective vaccine against Marek's disease.

1976
Avian leukosis virus antigens were detected in egg albumens of infected hens and detection procedures were applied in eradication programs.

1983
Embryonic vaccination was shown to be an effective approach in early immunization protocols for Marek's disease.

1983
Hemorrhagic enteritis virus of turkeys was successfully propagated in a cell line and a vaccine was developed.

1984-1986
Monoclonal antibodies against Marek's disease and avian reticuloendotheliosis viruses were developed.

1988
An endogenous avian leukosis virus was found to be closely associated with the sex-linked slow-feathering gene in White Leghorns.

1988
Transgenic chickens resistant to avian leukosis virus were generated through insertion of viral envelope genes into the host germ line.

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